

Neisseria species (such as *Neisseria meningitidis*), *Enterococcus* species (such as *Enterococcus faecalis*, *Enterobacter cloacae*) *Escherichia coli* and *Klebsiella pneumoniae*.

Example 7 - Typing of bacteria by dideoxy DNA-sequencing

The general approach of example 6 was repeated, and dideoxy (Sanger) DNA-sequencing was used as the sequencing method. The primer pool comprising 19b/31b (19b binds to *Listeria monocytogenes*, 31b binds to *Haemophilus influenzae*. The results show that Sanger sequencing is also suitable to use for analysis of typing with the set of primers of the invention. Fig 12 shows sequencing *Listeria monocytogenes* by sequencing primers of invention. An ABI prism DNA analyzer 3700 (Applied biosystems) was used for the DNA-sequencing with Big dye terminator kit according the manufacturer's manual. This work is in press in journal of Molecular and Cellular Probes.

Claims

My claims for the invention are:

1 The multiple sequencing oligonucleotide primer pool method is utilized for specific genotyping/typing/identification/detection/sequencing of a sample of nucleic acid molecules, whereby i) the molecules are suspected to contain at least one type/species/target of a target/variable region or region of interest, each type having different nucleotide patterns ii) the molecules are suspected to contain unspecific amplification in the amplification product and comprising the steps of:

- (a) Providing the sample is of nucleic acid molecules;
- (b) Providing a mixed set of at least two sequencing oligonucleotide primers, whereby each primer is designed for being specific for one type/species/group/target chosen from the known set of types/target of the nucleic acid sample, thereby allowing a primer, which is specific for a type/species/group/target that is present in the sample, to hybridize in or close to the target/variable region or region of interest;

- (c) Mixing the set of sample and primers under conditions allowing a primer or primers to hybridize if a type or types that is specific for the primer or primers is present in the sample;
- (d) Determining the type/species/target region to which the primer or primers has hybridized by extending the hybridized primer(s) in a DNA-sequencing reaction.
- 2 Method according to claim 1, wherein the sequencing reaction is performed by sequencing-by-synthesis, dideoxy sequencing (sanger), sequencing by mass spectrometry or any other DNA sequencing technology, applicable to the method of invention.
- 3 Method according to any one of claim 1-2, wherein the sample is a microorganism, such as a virus, fungi or bacteria.
- 4 Method according to any one of claim 1-3, wherein the sample is suspected to comprise at least two types/species/targets of nucleic acid molecules chosen from the known set of types/species/targets.
- 5 Method according to claim 4, wherein the sample contains multiple infection/variants/types/species.
- 6 Method according to claim 5, wherein at least one primer is specific for a variant of a disease linked to the microorganism.
- 7 Method according to claim 6, wherein the microorganism is a human papillomavirus (HPV)
- 8 Method according to claim 7, wherein the known set of HPV-types are chosen from the group comprising the HPV-types; high-risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 66, 68, 69 and low-risk: 6, 11, 34, 40, 42, 43, 44
- 9 Method according to any one of the preceding claims, wherein the sequence distance from the 3'-end of the general/consensus extendable primer could be avoided to be sequenced as in semi-conservative regions
- 10 Method according to any one of the preceding claims, for typing of amplicons comprising at least one semi-conservative region.
- 11 Method according to any one of claim 1-10 for typing of samples where a type/species is in minority or if the amplification fragment has low yield.
- 12 Method according to any one of claim 1-11, for typing/sequencing of samples containing unspecific amplification products, the primers of the set of primers not annealing to unspecific amplification products.

- 13 Kit for use in the method of typing of claim 1-12, comprising at least two oligonucleotide primers, whereby each primer is designed for being specific for one genotypes/type/species/group/target or type-specific region chosen from a known set of types of the type-specific/target region of a nucleic acid sample, thereby allowing a primer, which is specific for a genotype/type/species/target that is present in the sample, to hybridize in or close to the type-specific/target region or region of interest.
- 14 Kit according to claim 13, wherein the oligonucleotide primers of the kit are designed for being specific for any of the HPV-types chosen from the group comprising: high-risk: HPV-16, 18, 31, 33, 35, 39,45 , 51, 52, 58, 59, 66, 68, 69 and low-risk: HPV-6, 11, 34, 40, 42, 43, 44
- 15 Kit according to any one of the preceding claims for genotyping/typing/detection/identification/sequencing of any microorganisms, viruses or any other application where the multiple sequencing oligonucleotide primer pool approach is applicable by DNA sequencing technologies
- 16 Kit according to claim 1 for quantitative measurements of different genotypes/species/types amplified by PCR in the same sample or by mixing different amplicons

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